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Kinesin motor stepping challenged

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CHAPTER 5

SUMMARY

The cell is the smallest structural and functional unit of living organisms. The internal environment of the eukaryotic cell is very complex, dynamic and crowded with various cellular entities. Intracellular transport is necessary for maintaining proper cellular function in the eukaryotic cells. Failures in the active transport machinery can result in several types of fatal neurodegenerative diseases, like-amyotrophic lateral sclerosis (ALS) and Griscelli syndrome (GS). Transport within cells is accomplished through a combination of passive diffusion and active transport by molecular motors that convert the chemical energy of ATP into mechanical work, by moving in a hand-over-hand fashion along the complex network of proteinaceous cytoskeletal filaments. Long-range directed movement is powered by these nanoscale engines, which are critical for efficient delivery of cargos to specific locations. Numerous motor proteins with distinct motility properties transport a wide variety of cargoes, enable cell locomotion, drive cell division and also allow organisms to move. Although motor-based transport is known to be necessary to overcome cytoplasmic crowding and the limited range of diffusion within reasonable time scales, different aspects of motor protein traffic have not been fully understood yet, like how crowding of molecular motors along the cytoskeletal filaments affects transport efficiency. Like vehicle traffic congestions along highways (transport networks), molecular motors are expected to encounter traffic-jam-like situations along the cellular highways (the cytoskeleton network), but this has hardly been probed experimentally. Single-molecule motility assays and single-particle tracking methods can be applied to measure the velocity and run

length when the motors are well separated, these methods break down when the average distance between motors approaches the diffraction limit and then we need a technique through which we can study the crowding dynamics of molecular motors in *in vitro* or *in vivo* experimental systems.

In this thesis (Chapters 2 and 3), I have studied the dynamics of homodimeric Kinesin-1 from *Drosophilla*, homodimeric Kinesin-2 (OSM-3) and heterodimeric Kinesin-2 (Kinesin-II) from *C. elegans* under crowding conditions. The goal was to test whether these motor proteins with distinct motility parameters, have different strategies to overcome “traffic-jam” situations. I used a novel correlation image analysis technique to analyze image sequences acquired using Total Internal Reflection Fluorescence (TIRF) microscopy of fluorescently labeled motors at high densities. I measured motor velocities and run lengths at high motor densities, allowing a quantitative assessment of jamming effects in motor-protein transport. By comparing experimental results with model predictions, I arrived at a clear understanding of the collective motor-protein transport under crowding conditions, governed by motor-specific strategies.

Measurements of motor density, velocity and average run length along crowded microtubule filaments revealed remarkably different crowding behavior of Kinesin-1, OSM-3 and Kinesin-II motors. For Kinesin-1, the motility parameters are affected by molecular motor crowding at densities (~ 2 motors/ μm) indicates that motors start to interact when the average distance is $0.5 \mu\text{m}$, much larger than their physical size (16 nm) with maximum occupancy of ~ 10 motors/ μm , whereas velocity and run length of OSM-3 start decaying at much higher densities (~ 30 motors/ μm) corresponding to an average motor separation of 16 nm, and their maximum density of around 100 motors/ μm indicates multiple protofilament occupancy. Our studies on Kinesin-II showed that it is even less affected by crowding and show velocity and run length

decrease at a density of ~ 200 motors/ μm indicating almost 0 nm interaction length between the motors, and their maximum occupancy along a microtubule could reach up to around 400 motors/ μm . Altogether this study shows that the different motor proteins appear to be adapted differently to molecular motor traffic jam like situation, with Kinesin-II being least affected, substantially less so than OSM-3, which is from the same, Kinesin-2, family of kinesins.

These findings provide important insights in the functional specialization of the different kinesins. The combination of TASEP modeling and microscopic correlation imaging hence provides fundamental insight into the interactions of individual motors at the nanometer scale. Kinesin-1 motors typically work individually or are engaged in small teams (~ 1 -10) that may belong to the same or to different motor species, driving axonal transport of vesicles and organelles. OSM-3 and Kinesin-II together drive, in large teams (~ 50 or more), intraflagellar transport trains in the chemosensory cilia of *C. elegans*. It is tempting to speculate that the different interaction ranges found for the three motors play a key role in their efficiency to work in large teams. However, this area of research is still in its nascent stage and could be further explored in the future.

In chapter 4, I presented a systematic study of the impact of temperature on the motility properties of Kinesin-1, OSM-3 and Kinesin-II in single-molecule motility assays. Our understanding of the temperature dependency of motor stepping was limited and to some extent incomplete. Several studies employing multi-motor gliding assays have reported the impact of temperature on motor proteins, primarily Kinesin-1. However, the temperature changes the complete motor mechanochemistry and therefore, also run length and single-motor velocity, which had not been studied before. Since enzyme-catalyzed biochemical reactions, like the stepping of kinesins, can be kinetically complex, involving several subsequent rate-limiting steps, the relationship between

kinesin motility and temperature can be expected be more complex than simple Arrhenius kinetics. In chapter 4, I fitted the experimental results of the temperature dependence of single-motor velocity and average run length with kinetic models, providing new insights in the temperature-dependent mechanochemistry of Kinesin-1, OSM-3 and Kinesin-II.